SYMPOSIUM III

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MEMBRANE-CYTOSKELETON LINKAGES AT FOCAL CONTACTS: ROLE IN SUBSTRATUM ADHESION AND SIGNAL TRANSDUCTION

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Animal cells associate with extracellular matrix components at specialized regions of the plasma membrane called adhesion plaques or focal contacts. At these sites, extracellular matrix receptors that are members of the integrin family of cell adhesion molecules mediate a transmembrane connection between elements of the extracellular matrix and the actin cytoskeleton. The establishment of a link between matrix and the cytoskeleton is essential for stable cell-substratum attachment. Cell adhesion to extracellular matrix can trigger a variety of responses by cells including changes in cell morphology, migratory activity, growth properties, and gene expression. Regulatory proteins including the calcium-dependent protease II, protein kinase C, the focal adhesion kinase (FAK), and a number of proto-oncogene encoded tyrosine kinases are concentrated at adhesion plaques and have been postulated to participate in signal transduction events at the adhesive membrane. Moreover, we have recently identified and characterized a low abundance adhesion plaque protein called zyxin (1-3), which displays three copies of the LIM motif, a "zinc-finger" domain that is also found in a variety of proteins implicated in the regulation of gene expression during development. The LIM motif is evolutionarily conserved and has been identified in proteins from mammals, birds, and amphibians as well as invertebrates and plants. Our recent studies on the structure and function of cytoskeletal LIM-proteins such as zyxin will be described.

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MOLECULAR GENETIC ANALYSIS OF THE MICROTUBULE CYTOSKELETON IN PLANTS

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Microtubules, filaments composed of α- and β-tubulin, play important roles in plant morphogenesis. Both cell division and cell elongation processes are dependent on dynamic microtubule arrays including the cortical microtubules found in interphase cells, and the preprophase band, mitotic spindle, and phragmoplast microtubules in dividing cells.

A variety of tubulin isotypes are expressed during plant development. In order to better understand the genetic information encoding tubulin in higher plants, our groups have examined the structure and expression of the genes/cDNA clones encoding α-tubulin and β-tubulin in the dicot *Arabidopsis thaliana* and in the monocot *Zea mays*. In the small genome of *Arabidopsis*, we detected and characterized six α-tubulin genes and nine β-tubulin genes, all of which are expressed. The tubulin gene families in maize are even larger, with an estimated eight α-tubulin genes and 12-15 β-tubulin genes. Phylogenetic analyses showed that the α-tubulin genes in both *Arabidopsis* and maize were derived from two ancient α-tubulin genes that predate the divergence of monocots and dicots.

The spatial and temporal patterns of gene expression have been studied using RNA hybridization with gene-specific probes. Results from these studies showed that most genes are expressed in all or most tissues, but that each gene has a unique pattern of expression. Several genes exhibit preferential expression in certain tissues, which we have examined in *Arabidopsis* transgenic plants containing fusion genes constructed from tubulin gene promoters and the β-glucuronidase coding region. For example, the *TUA1* gene of *Arabidopsis*, which encodes the most divergent α-tubulin isoform, is expressed primarily in pollen which has completed the mitotic divisions. To learn more about the functional significance of multiple tubulin isoforms, we have generated isoform-specific antibodies to examine the distribution of these isoforms in different tissues. Results from immunofluorescence localization experiments suggest that most isoforms can assemble into more than one microtubule array within a cell, supporting the notion that multiple tubulin genes are most important for regulating the overall production of tubulin.

Microtubule assembly and function in cells is controlled by proteins that interact with microtubules. To identify components that may participate in nucleating microtubule assembly in plant cells, we have examined two *Arabidopsis* genes encoding γ-tubulin, a protein involved in nucleating microtubule assembly in animal and fungal cells.

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ADHESION OF THE PLASMA MEMBRANE TO THE CELL WALL IN RESPONSE TO ENVIRONMENTAL AND PATHOGENIC STRESSES

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One consequence of adaptation to environmental or pathogen stress is a formation of tight adhesion of the plasma membrane to cell wall. Protoplasts isolated from salt-adapted cells retain the complementary adhesive characteristic and adhere tightly to each other, whereas protoplasts from unadapted cells do not. The hexapeptide gly-*arg-gly-asp*-ser-pro specifically disrupts self-adhesion of protoplasts from salt-adapted tobacco cells. The tripeptide sequence arg-gly-asp represents the integrin binding domain of several animal extracellular matrix proteins such as fibronectin (Fn) and vitronectin (Vn). Proteins immunologically related to human Vn and Fn are enriched in cell walls and membranes of salt-adapted tobacco suspension culture cells [Zhu *et al.*, Plant Journal, *in press*]. Transmembrane linkers are also important constituents of an "adhesion complex" that forms a continuum between the cell wall and cytoskeleton. An induced resistance response in cereals is accompanied by expression of a gene encoding an 11 kDa putative transmembrane protein. Sequence analysis of known glycine-rich proteins (GRP's) indicates that some GRP's may form a structural plate inside the plasma membrane. These GRP's may function as one possible site of attachment of the membrane to the cytoskeleton. We propose that induced adhesion of the plasma membrane to the cell wall is not just a phenomenological curiosity but a visual marker of the continuum of the cytoskeleton, through the plasma membrane to the cell wall. Integrated with this molecular scaffolding could reside the dynamic components of environmental perception, signal transduction, and the elements of cellular responses to stress.

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THE CONTRIBUTION OF THE CELL-EXTRACELLULAR MATRIX JUNCTION TO GRAVISENSING IN CHARA

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When an internodal cell of Chara is oriented vertically, the cytoplasm streams down 10% faster than it streams up (1). However, when the cells are treated with various impermeant proteases that act at the plasma membrane-extracellular matrix junction, gravisensing is inhibited even though streaming continues. The gravireceptor is sensitive to Proteinase K, thermolysin and collagenase, but not to trypsin, α-chymotrypsin or carboxypeptidase B. Moreover, the tetrapeptide RGDS inhibits gravisensing in a concentration-dependent manner, indicating that the gravireceptor may be an integrin-like protein. Through localized treatments, we have determined that the gravireceptor protein is localized at the ends of the cell (2).

Further evidence that the gravireceptor is localized at the plasma membrane-extracellular matrix junctions at the ends of the cell comes from the observation that a unidirectionally-applied hydrostatic pressure (490 Pa) mimics the effect of gravity in inducing a polarity of cytoplasmic streaming. The pressure-induced polarity has identical requirements and sensitivities to inhibitors as the gravity-induced polarity (3).

Lastly, the magnitude and the sign of the graviresponse is modulated by the external $[Ca^{2+}]$. The Ca^{2+} required for gravisensing appears to enter the cell through two classes of channels that are localized at the ends of the cells. Each one is pharmacologically distinct from the class of Ca^{2+} channels that is involved in E-C coupling and is localized along the flanks of the cell. We have not yet determined the relationship between the gravireceptor protein and the channels.

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SYMPOSIUM III

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VITRONECTIN-LIKE PROTEINS IN FUCUS EMBRYOS AND OTHER PLANTS.
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The rhizoid cell of the two-celled Fucus embryo attaches the developing embryo to the substratum. The rhizoid cell is highly polar and directionally transports (via Golgi vesicles) macromolecules that are secreted into the elongating cell wall or extracellular matrix (ECM). Previous results indicate that a highly sulfated fucan glycoprotein (F2) is locally deposited in the ECM of this elongating tip. If F2 is prevented from being enzymatically sulfated (met-embryos), normal two-celled embryos are formed but F2 is not localized in the rhizoid tip and the embryos do not adhere. Recent results have identified a vitronectin-like (Vnl) molecule with the same pattern of distribution as F2 in the Fucus two-celled embryo. Polyclonal antibodies to human vitronectin recognize a glycoprotein in extracts of zygotes and two-celled embryos of Fucus with a molecular weight (62-65 kDa) similar to human vitronectin (Vn). The specificity of the immuno-cross-reactivity is further demonstrated using controls of monospecific and non-immune antibodies, and competition by purified Vn. The Fucus Vnl molecule can also be isolated by procedures established for mammalian Vn; glass bead and heparin affinity chromatography. Immunolocalization and subcellular fractionation results demonstrate that Vnl is first localized in the cytoplasm of the zygote and later in the ECM of the elongating rhizoid tip. Met-embryos do not exhibit this localized ECM distribution of Vnl and do not adhere to the substratum (similar to the results with F2). In a functional assay, adhesion of Fucus embryos is prevented in the presence of the Vn antibody, suggesting that the Vnl plays a role in adhesion as in animal systems (1,2). Progress on the isolation of genes from Fucus that code for Vnl proteins will be presented. Evidence for the presence of Vnl proteins in fungi, algae and higher plants will be presented and their possible physiological roles will be discussed (3,4). Supported by research grants to R.S.Q. from the N. S. F. (DCB-8917540) and the Office of Naval Research (N00014-91-J-4128).

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DEVELOPING A TRANSGENIC PLANT MODEL FOR SELF-INCOMPATIBILITY IN THE SOLANACEAE

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Self-incompatibility (SI) in solanaceous plants involves the recognition and rejection of self-pollen. The process is controlled by a poly-allelic genetic locus, the *S*-locus. Rejection occurs when a haploid pollen tube contains an allele of the *S*-locus identical to one of the two *S*-alleles present in the diploid pistil. Our interest is in how the products of the *S*-locus condition *S*-allele specific pollen rejection. Along with several other groups, we have cloned cDNAs and genomic sequences corresponding to pistil specific products of the S-locus, the *S*-RNases. These proteins are thought to exert a cytotoxic effect that accounts for *S*-allele specific rejection of pollen.

The focus of our work is to reproduce *S*-allele specific pollen rejection in a transgenic plant system. Eventually, this will allow us to determine the effects of specific changes in *S*-RNase sequences on pollination behavior. Our approach is to fuse *S*-RNase sequences to heterologous promoters followed by transformation into plants with an appropriate genetic background. Previous results suggest that achieving high level expression in the mature transmitting tract will be the major barrier to achieving this goal. Therefore, our constructs are designed for optimum expression in this tissue. We will present results of transformation experiments involving several different viral and plant promoters as well as different construction strategies.

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EXPRESSION OF SOYBEAN A ASPARAGINE SYNTHETASE GENE IN ESCHERICHIA COLI K-12

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Asparagine plays a significant role in plant growth and development because of its involvement as a nitrogen transport compound. Asparagine biosynthesis is mediated by the enzyme asparagine synthetase (AS). AS has been extremely difficult to characterize due to its instability in vitro. Therefore, expression of a soybean AS gene in E. coli would enable the purification of the AS protein for characterization and antibody production. The region corresponding to the predicted mature soybean asparagine synthetase (SAS2) protein was amplified by the polymerase chain reaction (PCR). The 5' oligonucleotide primer contained the proposed glutamine binding site with an EcoRI site at the extreme 5' end. The inverse oligonucleotide primer corresponded to a region 3' of the stop codon along with a BamHI site. The primers were designed to place SAS2 gene in-frame with the lacZ gene of the pUC18 vector. Plasmid DNA from the positive colonies was isolated and used to transform an AS deficient E. coli strain. Complementation experiments revealed that the soybean AS gene was expressed functionally in E. coli. This was confirmed in preliminary AS assays using the HPLC which directly measured the synthesis of asparagine. Future experiments will included optimizing the AS assay and purification of the AS protein.

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CLONING AND EXPRESSION OF THE SOYBEAN LYSINE PATHWAY GENE DAPA

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In plants, the first committed step in the pathway to lysine synthesis is the condensation of aspartate B-semialdehyde and pyruvate, catalyzed by the enzyme dihydrodipicolinate synthase (DS). DS activity undergoes feedback inhibition by lysine, suggesting that the regulation of DS activity may control lysine synthesis. Cloning of the soybean dapA gene is of potential economic importance because soybeans are a major agronomic source of lysine, and the genetic engineering of the dapA gene may be an important way of increasing lysine synthesis in soybeans and other crop plants. The gene which encodes DS has been cloned from monocots, but it has not been previously cloned from dicots. We amplified a portion of the soybean (G. max var Century) DS gene using PCR. The DS gene fragment was cloned and used to isolate copies of the gene from genomic DNA and cDNA libraries as well as 3' RACE reactions. dapA cDNA was synthesized using a dapA specific 3' primer, and the coding region of the mature DS protein was amplified using PCR for the expression in pUC18. We have also sequenced more than 1 kb of genomic DNA 5' to the sequences encoding mature DS.

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GENETIC REARRANGEMENT AND DISTRIBUTION OF REPEATED SEQUENCES SURROUNDING DUPLICATE A71 LOCI IN SOYBEAN

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The soybean genome contains many duplicate sequences due to tetraploidization of the progenitor genome. RFLP mapping indicates that subsequent processes related to diploidization resulted in significant rearrangement and scrambling of these sequences. To determine the extent of scrambling and the types of rearrangements which have occurred we have begun molecular comparisons of duplicate loci. Probe pA71 detects at least ten fragments in a Southern hybridization of *TaqI* digested soybean DNA. Seven of these fragments were isolated as independent λ genomic clones. Two groups were identified whose members appeared to be related based on similarity of restriction sites and cross-hybridization of regions outside the pA71-hybridizing region. One group (two clones) exhibited a region of homology beginning within and extending to the end of the λ clones. The second group (four clones) exhibited a more complex cross-hybridization pattern of homologous regions interspersed with non-homologous regions. Cross-hybridizing regions were assayed for co-linearity or rearrangement by subcloning the cross-hybridizing regions and determining where each subclone cross-hybridized to other group members. Each clone was also assayed for the presence of repeated sequences in order to determine whether the distribution of such sequences was different between homologous regions and to determine the distribution of these sequences in the soybean genome.

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MECHANISM OF ANTISENSE INHIBITION IN TOBACCO ANTISENSE MUTANTS

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Antisense technology is fast becoming an integral tool for molecular biologists in studying the *in vivo* effects of specific genes, yet in plants the mechanism by which this inhibition operates is not fully understood. Using two antisense tobacco mutants, one expressing *rbcS* antisense mRNA sequences and the other expressing Rubisco activase antisense mRNA sequences, we are attempting to better understand this mechanism. Both of the antisense tobacco mutants show a decrease in mRNA levels for the target gene. This indicates that the antisense RNA acts either to interrupt transcription of the mRNA or acts to decrease the mRNA's stability once it is produced. To differentiate between the two we performed nuclear runoff transcription experiments. These experiments showed that the level of transcription of the target mRNA was similar in both the antisense and wildtype plants. These results indicate that the antisense mRNA doesn't act at the level of transcription. To see if the decreased stability hypothesis is correct we are conducting mRNA half-life experiments and use PCR to check for the theoretical double stranded RNA intermediate. Results of these experiments will be presented.

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THE *Arab6* GENE IN *Arabidopsis thaliana* ENCODES A SMALL GTP-BINDING PROTEIN.

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GTP-binding proteins are fundamental components of the biochemical complexes that regulate the transport of vesicles through the exocytic and endocytotic pathway of eukaryotic cells. Although extensively studied in yeast and mammalian systems, few homologues have been described in plants. We have isolated, cloned and characterized a cDNA from *Arabidopsis thaliana*, denoted *Arab6*, encoding a protein related to ryh1 of *Schizosaccharomyces pombe* and mammalian rab6. The *Arab6* clone contains an open reading frame encoding a polypeptide of 208 amino acids and a molecular mass of 23,129 daltons. *Arab6* shows 78% amino acid identity to rhy1 and shares 79% homology to rab6 and, therefore, may be considered yeast and mammalian counterparts, respectively. Northern blot analysis revealed the presence of the *Arab6* RNA in leaves, roots, flowers, and 2- and 4-day-old seedlings. To examine the role of *Arab6* in intracellular protein trafficking in plants we have made specific amino acid changes in a GTP-binding domain (N^{121}-->I^{121}) as well as at the C-terminal lipidation site required for membrane attachment. The capacity of bacterial overexpressed *Arab6* and *Arab6*-mutant proteins for GTP-binding was analyzed on protein blots. This analysis revealed that purified wild-type *Arab6* protein and the C-terminal mutant retained GTP-binding capability, while the N^{121}->I^{121} mutation rendered the protein incapable of binding GTP. All of the mutants produced proteins that were recognized by antibodies to the wild-type protein. Future research will focus on the immunolocalization of the wild-type and mutant proteins in stably transformed plants.

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ISOLATION AND EXPRESSION OF A TRYPTOPHAN DECARBOXYLASE GENE FROM CAMPTOTHECA ACUMINATA

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The indole alkaloid camptothecin and its derivative 10-hydroxy camptothecin, are currently in clinical trials for the treatment of a variety of cancers. Presently, these drugs are extracted from the bark of the chinese wonder tree, Camptotheca acuminata. Using a heterologous probe from Catharanthus roseus (from V. De Luca, Univ. of Montreal) we have isolated a gene for tryptophan decarboxylase (TDC) from Camptotheca. TDC is the first committed step in indole alkaloid biosynthesis and therefore represents a potential "bottleneck" enzyme in the pathway. Sequence comparisons show that the Camptotheca TDC is highly homologous to other aromatic amino acid decarboxylases and contains a conserved pyridoxal phosphate binding domain. RNA gel blot analysis indicates that TDC is expressed in whole 2-week old seedlings, but is only detectable in the bark of larger trees, and not in leaves or roots. Treatment of whole plants with 100 uM methyl jasmonate for 24 hour stimulated TDC expression in leaves. These results may have implications for the production of Camptotheca alkaloids in response to pathogen elicitation.

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PHYSICAL MAPPING OF A 470 KB CHROMOSOMAL SEGMENT OF THE MAIZE GENOME CONTAINING *A1* AND *SH2* GENES.

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The analysis of the organizaton and structure of complex genomes can be greatly eased by the utilization of yeast artificial chromosomes (YACs). We are characterizing a YAC composed of a 470 kb fragment from the long arm of chromosome 3 of maize that contains *A1* and *Sh2* genes. The genetic distance between *A1* and *Sh2* is 0.1 cM. Combining genetic analysis and a YAC fragmentation technique (Pavan et al., 1991) we have determined the physical distance between *A1* and *Sh2* (about 140 kb) and their relative orientations on the maize chromosome. We are currently investigating the distribution of single-copy and repetitive DNA sequences together with the identification of expressed genes in this region of the maize genome. The availability of single-copy sequences will enable us to use them as RFLP probes in order to identify meiotic recombination sites in the region between *A1* and *Sh2*.

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CHARACTERIZATION OF TWO CDNAS ENCODING CYSTEINE-RICH PROTEINS EXPRESSED PREFERENTIALLY IN TOMATO STAMENS

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The tapetum is a secretory tissue that surrounds the pollen sac. It has been proposed to have the essential functions of supplying nutrition to the pollen grains, dissolution of tetrads through the production of callase, and synthesis of materials for the exine layer of the pollen. A first step in the analysis of the function of the tapetum in microsporogenesis was the isolation of genes with tapetal-specific expression. Toward this end two cDNA clones, TomA5B and TomA108Z, encoding cysteine-rich proteins were isolated and characterized. The genes are single copy in the tomato genome. Expression is specific to the tapetal cells from pre-meiosis through tetrad release. The deduced proteins have an N-terminal hydrophobic domain suggesting that they are secreted proteins. The pattern of cysteine residues of the deduced proteins is very similar to that found in other stamen-specific genes and to a superfamily of seed proteins that consists of protease inhibitors, α-amylase inhibitors, and seed storage proteins. There is amino acid sequence similarity between the 108 protein and other stamen-specific genes. However, there is no similarity between the 5B protein and the stamen-specific genes or the seed proteins beyond the spacing of cysteines. The possible functions of these proteins will be discussed.

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MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF A PUTATIVE GLUTATHIONE PEROXIDASE GENE FROM CITRUS

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Cit-SAP is a salt stress associated protein that was originally identified in salt tolerant cultured cells from Shamuti orange (*Citrus sinensis* L.Osbeck). The gene for the protein (*csa*) was isolated and its primary nucleotide sequence was determined. The predicted amino acid sequence agrees well with partial amino acid sequence directly determined from the purified protein. *csa* is highly homologous to a tobacco gene that is expressed in stressed protoplasts. A marked overlap is observed between the amino acid sequence of Cit-SAP and that from a mammalian glutathione peroxidase. High homology between the amino acids is observed particularly at the region corresponding to the active site of the enzyme. The expression of the citrus *csa* gene in *E. coli* clearly confers higher tolerance to paraquat, an O_2^- radical forming agent. This data supports the assumption that Cit-SAP functions as a plant glutathione peroxidase and might be a part of the plant oxygen free radicals scavenging system. The promoter region of *csa* is now being analyzed in order to understand the mode of regulation of *csa*. *Agrobacterium* vectors designed to over-express the sense and anti-sense gene orientations were constructed and are now used to study the function of *csa* in stressed plants.

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THE PHOTOSYNTHETIC NITROGEN-FIXING MICROSYMBIONT *BRADYRHIZOBIUM*, STRAIN BTAi 1: LIGHT QUALITY AND INTENSITY EFFECTS ON BACTERIOCHLOROPHYLL PRODUCTION AND ACCUMULATION

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Numerous strains of a new exciting class of photosynthetic nitrogen-fixing rhizobia, now identified as members of the genus *Bradyrhizobium*, have been isolated from several species of the stem and root nodulating host *Aeschynomene*. Common characteristics of these bradyrhizobia include symbiotic nitrogen fixation, CO_2 uptake, production of carotenoids and bacteriochlorophyll (BChl), assemblage of photosynthetic reaction centers, and a photoperiod requirement for maximum pigment production. We have investigated this unusual photoperiod requirement in strain BTAi 1. BChl accumulates in the dark during the light/dark photoperiod, following light initiation, with no net accumulation in the light. A light initiation of at least 30 minutes is required for detectable levels of BChl accumulation and 24 hours for optimal accumulation. The action spectrum for BChl accumulation shows a response in the blue but not in the far red, indicating a photoreceptor other than BChl itself. This photoreceptor responds at low light intensity.

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INTERACTIONS IN THE RHIZOSHEATH

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Rhizosheaths are encasements of aggregated soil found tightly bound to the younger regions of grass roots. The components involved in their formation and stabilization include soil particles, adhesive mucilages produced by both root-cap cells and native bacteria, root hairs and water. In the sheathed portion of a root, the large, late-metaxylem elements are closed. Therefore, this young region has a low capacity for axial conductance of water (McCully and Canny, 1988, Plant Soil 111: 3-14), and has a higher water content than the older, sheath-free (bare) portion (Wang *et al.*, 1991, Physiol. Plant. 82: 157-162). Interestingly, despite their relatively high water status, heavily-sheathed roots are reported in xerophytic grasses. Preliminary observations of mesophytic grasses growing in dry soils indicate their roots to have more substantial and tightly-bound rhizosheaths than similar roots growing in wetter soils. We have developed a system (modified from Nambiar, 1976, Plant Soil 42: 267-271) to quantify the relationship between the formation and stabilization of rhizosheaths, and the soil water content. Young corn roots are grown through individual columns of soil each with an isolated zone of soil (sandwiched by two wax membranes) of controlled water status. Measurements are presented of sheath/root volumes and soil dry weights of fractions removed by specific treatments (sonication, 60°C water, and gentle abrasion) from the controlled zone of the root. These show more soil, more tightly bound at low water status, and less soil, more weakly bound in wetter soils. The role of water in the binding mechanisms of the rhizosheath, and, more importantly, the source of this water, especially in dry soils, will be discussed.

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SALICYLIC ACID BIOSYNTHESIS IN VIRUS-INOCULATED TOBACCO

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Salicylic acid (SA) is a likely endogenous regulator of localized and systemic disease resistance in plants. During the hypersensitive response (HR) of Xanthi-nc tobacco to tobacco mosaic virus (TMV) SA levels rise dramatically. This rise induces accumulation of pathogenesis-related (PR) proteins and increases resistance to a subsequent challenge by pathogens. To study SA biosynthesis we monitored the levels of SA and its likely precursors in extracts of tobacco leaves and cell suspensions by HPLC linked to fluorescence, radioactivity and UV detectors. Radio tracer studies demonstrated that SA is synthesized from cinnamic acid (CA) via benzoic (BA). 2-hydroxycinnamic acid (2HCA) did not function as a SA precursor in tobacco. The increase in SA during development of the HR was associated with a rise in BA levels. In suspension-cultured tobacco cells, and TMV-inoculated Xanthi-nc tobacco leaves BA, but not 2HCA induced SA accumulation. Consistent with BA being the immediate precursor of SA, we observed induction of PR-1 protein accumulation and increased resistance to TMV in BA- but not in 2HCA-treated tobacco leaves.

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VIRAL INFECTION INDUCES BENZOIC ACID 2-HYDROXYLASE ACTIVITY IN TOBACCO

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Salicylic acid (SA) is a likely signal which activates plant resistance to pathogens. Tobacco leaf extracts contained benzoic acid 2-hydroxylase (BA2H) activity responsible for the 2-hydroxylation of benzoic acid (BA) to SA. This enzyme required NAD(P)H or reduced methyl viologen as electron donors and was induced by tobacco mosaic virus (TMV) inoculation. At 24 °C the induction of BA2H activity was due to *de novo* protein synthesis and paralleled an increase in free SA in tissues expressing hypersensitive response to TMV. At 32 °C TMV failed to induce BA2H activity and SA increases. However, when the inoculated plants were transferred to 24 °C, a 15-fold BA2H induction was observed. This increase in enzyme activity produced a 65-fold increase in SA content over the basal levels 12 h after temperature shift. The effect of TMV infection could be duplicated by infiltrating healthy tobacco leaf tissue with BA at concentrations similar to those observed following TMV inoculation. This induction was specific to BA. Neither phenylalanine nor cinnamic or *o*-coumaric acids induced BA2H.

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EVIDENCE OF A ROLE OF LIPOXYGENASE PRODUCTS IN PLANT DEFENSE RESPONSES

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It has been suggested that release of polyunsaturated fatty acids from glycerolipids is a controlling factor in the formation of fatty acid peroxidation products such as aldehydes. Such products derived from fatty acid peroxidation include the carbonyl compounds, traumatin, jasmonic acid and volatile aldehydes. Production of elevated levels of these compounds may in turn enhance plant pest defenses. Infiltration of plant leaves with lipoxygenase substrates increased formation of such compounds and elicited some phenomena associated with the hypersensitive response (HR) such as electrolyte leakage and necrosis. Transgenic plants with elevated lipoxygenase activity were induced to form correspondingly higher levels of fatty acid peroxidation products. Non-lipoxygenase substrate fatty acids had no effect. Heat treatment of leaves or application of lipoxygenase inhibitors greatly reduced or eliminated linoleic or linolenic acid induced increases in peroxidation product formation. Aldehydes resulting from lipoxygenase/hydroperoxide lyase action were found to cause electrolyte leakage and necrosis. Exposure to methyl jasmonate for 3 days also induced electrolyte leakage and chlorosis.

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PATHOGENESIS RELATED PROTEINS INDUCED BY MICROBOTRYUM VIOLACEUM IN SILENE LATIFOLIA.

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Strains of Silene latifolia, susceptible and resistant to the anther smut fungus Microbotryum violaceum, were analyzed for the pathogenesis related proteins (PRPs) ß-endoglucanase and chitinase. Total activities of the PRPs in vacuum infiltrates from leaves, stems and buds of infected and non-infected plants did not differ in the susceptible and resistant strains. However, gel electrophoresis of infiltrates from buds but not from stems and leaves of diseased susceptible and resistant strains showed significant differences in the PRP-isozyme patterns. We conclude that the PRP response is confined to the site of expression of the fungal pathogenesis and is not systemic in S. latifolia. PRPs were also detected in culture filtrates of cell suspension cultures from S. latifolia. Supported by grants from the Richter and Abbott Funds.

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CHARACTERIZATION OF FUNGAL GROWTH INHIBITION BY TAXANES

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Taxol, a diterpene amide isolated from *Taxus* spp., has been approved by FDA for the treatment of ovarian cancer. Little is known about the biosynthesis and biological role of this and related compounds. We have found that taxol and cephalomannine are active *in vitro* against various fungi which cause root-rot disease, such as *Phytophthora lateralis, P. dreschleri, P. cinnamomi,* and *Rhizoctonia solani.* Hyphal elongation of *P. lateralis,* the causal agent of Port-Orford-Cedar root-rot, was inhibited at 1 nmol on solid medium and at 10 nM in liquid medium. Growth of the other fungi was inhibited at 0.1-10 µM. In contrast, baccatin III did not show antifungal activity against these root pathogens. We are currently characterizing the growth and inhibition kinetics of the above fungi, mechanism of action, and the effect of taxol and taxanes on other classes of fungi. This work is supported in part by USDA, NSF, and the Charles A. Lindbergh Fund.

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THE ROLE OF SUCROSE INVERTASE IN WHEAT ROOTS ASSOCIATED WITH AMMONIA-EXCRETING MUTANT OF CYANOBACTERIUM *ANABAENA VARIABILIS*

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Wildtype nitrogen-fixing, filamentous cyanobacteria associate with wheat roots under favorable conditions, but they do not usually enhance the growth of wheat plants. The association of ammonia-excreting mutants of *A. variabilis* with wheat roots produces increases in biomass, grain yield, and total N-content of wheat. Sucrose is the main photoassimilate translocated in wheat leaves, however, ammonia-excreting strain SA-1 cannot use sucrose for growth in free-living conditions. ^{14}C-sucrose was translocated from wheat leaves to the roots in 30 min and one fourth of ^{14}C label was incorporated into strain SA-1. Wheat roots contain the enzyme ß-fructosidase (invertase) that cleaves sucrose into glucose and fructose. The levels of both hexoses were very low in media of the wheat/SA-1 co-cultures, where strain SA-1 was able to use added fructose, produced by invertase activity. Since less than 10% of invertase was excreted in nitrate-grown control plants, adsorption of the mutant to the roots was necessary for high ammonia production by strain SA-1 and maximal wheat growth.

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G-PROTEIN MEDIATED PLANT DEFENSE RESPONSE TO A CULTIVAR-SPECIFIC FUNGAL PATHOGEN.

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The resistance or susceptibility of host plants to different races of a pathogen is determined by the match of dominant resistance genes in the plant with dominant avirulence genes (*avr* genes) in the fungus. The induction of defense responses (incompatible interaction) is thought to be determined by specific host-pathogen recognition. We have previously reported that a race-specific elicitor, the putative product of an avirulence gene of *Cladosporium fulvum*, induced rapid changes at the surface of tomato cell suspensions carrying the complementary resistance gene. Here, we show that de-phosphorylation of the host plasma membrane ATPase, with a concomitant acidification of the extracellular matrix and the formation of callose, is an early component in the incompatible reaction between tomato and *Cladosporium fulvum*. Furthermore we demonstrate that a signal, initiated *via* the recognition of the elicitor by plant membrane-associated receptor(s) and leading to the activation of membrane-bound phosphatases, is transduced by G-proteins.

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GROOVES BETWEEN ROOT EPIDERMAL CELLS: ARE THESE A NEGLECTED FEATURE IMPORTANT IN ROOT FUNCTION?

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In surface view, the epidermal cells of a young root lie in sharply-defined, longitudinal files. This arrangement of the turgid cells resembles in form parallel rows of strings of long sausages. Grooves form between all the cells, but these are particularly wide and deep between adjacent files of cells. There are many reports of concentrations of rhizobacteria within these deep grooves, suggesting that they are sites of exudation. Recent studies by Varney and Canny (*New Phytologist*, in press) indicate that these regions in branch roots are also sites of high water flux into roots. Root epidermal development has been followed in the grasses. The deeply corrugated surface appears in the root hair zone. Here, a thick pellicle, which has overlain the younger epidermis and kept the cells tightly joined disintegrates. The cells retain a thick outer wall, but the loss of the pellicle allows them to bulge outward and the longitudinal files to separate. This separation exposes the middle lamella region of the outer ends of the thin radial walls which are rich in plasmodesmatal connections. We propose that this opening of the epidermis may provide a major path for exchange of material between root and soil.

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ACTIVATION OF ARABIDOPSIS K^+ CHANNELS BY ATP

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We are investigating the light-regulation of ion channels in the plasma membrane of mesophyll cells of *Arabidopsis thaliana*. Photosynthetically active light transiently depolarizes the plasma membrane of mesophyll cells in an intact leaf; however, when photosynthesis is blocked by either DCMU or the albino mutation *alb1*, no voltage transients occur upon illumination. These results indicate that the changes in membrane potential induced by light are mediated by photosynthesis. Of the three distinct cation channels that we are able to identify by I-V analysis, at least one K^+ channel (PKC1) is activated by light in attached but not excised patches. Since V_m is frequently more negative than E_K, increased opening of K^+ channels in light would contribute to depolarizing the membrane. Concentrations of ATP in the cytosolic range applied to the cytoplasmic side of an excised patch activate all three types of channels. Our results provide support for the hypothesis that ion channels in the plasma membrane are regulated by ATP produced by illuminated chloroplasts.

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DESIGN OF A "GENERIC" K CHANNEL ANTIBODY

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We designed a probe able to identify K channels by raising antibodies to a peptide (TLTTVGYGD) corresponding to the pore region which is highly conserved in a wide range of K channel proteins. A series of Western blot analyses were undertaken to evaluate whether the antibodies raised would cross react with K channels. Our antibody reacted with the 38 kd K channel in rat brain, showed no binding to acetylcholine receptor (an ion channel which conducts K and other ions but does not have the K channel pore sequence), but identified putative K channels in root plasmalemma, the chloroplast inner envelope, and in bovine brain which is enriched in K channels. Evidence that our preparation specifically reacted with K channels was obtained by probing cultures of kidney cells transfected with the DRK1 K channel gene, and yeast transfected with the (Arabidopsis) KAT1 gene. In both cases, our antibody reacted with a single protein expressed in the transfected cells, and not in the native cell lines. The protein expressed by yeast transfected with KAT1 had a MW of ≈80 kd which is similar to the MW deduced from the KAT1 gene sequence. We conclude that this antibody can be useful in identifying K channels in a broad range of membranes. Supported by USDA grant 92-01422 and a Johnson&Johnson Discovery grant.

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MOLECULAR AND BIOPHYSICAL ANALYSIS OF PLANT K^+ TRANSPORTERS

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Potassium (K^+) plays a vital role in many processes central to plant growth and development. Despite extensive studies of K^+ uptake and movement throughout the plant, the mechanisms by which K^+ is transported are poorly understood. The recent cloning of two plant K^+ transport genes, *KAT1* and *AKT1*, from *Arabidopsis thaliana* opens up new avenues of research in this field. Expression studies of these genes and biophysical analysis of the proteins that they encode are being conducted in an attempt to more thoroughly understand plant K^+ transport, with an emphasis on the identification of transport genes involved in root K^+ absorption. Northern analysis indicates that *KAT1* and *AKT1* exhibit strikingly different spatial regulation within the plant. Furthermore, additional K^+ transport genes appear to be present in the *Arabidopsis* genome. The biophysical studies involve the characterization and comparison of K^+ transport via *KAT1* and *AKT1* expressed in *Xenopus* oocytes. The use of this heterologous system has enabled us to study, in detail, the transport properties of the proteins encoded by *KAT1* and *AKT1*. Our findings suggest that K^+ transport within the plant is directed by differential regulation of a family of genes rather than modification of a single transport system.

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CONTROL OF IRON(III) AND COPPER(II) REDUCTION IN PEA ROOTS BY FE AND CU STATUS: DOES THE ROOT-CELL PLASMALEMMA FE(III)-CHELATE REDUCTASE PERFORM A GENERAL ROLE IN REGULATING CATION UPTAKE?

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The root-cell plasma membrane Fe(III)-chelate reductase plays a central role in Fe(III)-chelate reduction and subsequent Fe^{2+} uptake in dicots and nongraminaceous monocots. The role of Fe and Cu status in pea (*Pisum sativum* L.) seedlings in the regulation of this reductase was studied. Additionally, the ability of this reductase system to reduce Cu(II)-chelates as well as Fe(III)-chelates was investigated. Pea seedlings were grown in full nutrient solutions under control, -Fe, and -Cu conditions. Reduction of Fe(III)- and Cu(II)-chelates was visualized along roots submerged in agarose, and root reductase activity was quantified via spectrophotometric assay of the Fe(II)-BPDS and Cu(I)-BCDS chromophores. Reductase activity was stimulated in response to either Fe deficiency or Cu depletion of the seedlings. Roots from both Fe-deficient and Cu-deficient plants were able to reduce exogenous Cu(II)-chelate as well as Fe(III)-chelate. When reductase activity was stimulated by Fe deficiency, the absorption of a number of mineral cations (i.e., Cu, Mn, Fe, Mg) was significantly increased. In addition to playing a critical role in Fe absorption, plasma membrane reductases might play a general role in the regulation of root cation absorption, possibly via controlling the reduction of critical sulfhydryls in transport proteins involved in divalent cation transport (divalent cation channels?) across the root-cell plasmalemma.

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LIGHT-STIMULATED ION FLUX IN EPIDERMIS AND MESOPHYLL OF GROWING PEA LEAVES

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The stimulatory effect of light on leaf growth is perceived separately by epidermal and mesophyll cells. Light acts via phytochrome and a blue-light receptor, and in green cells, photosynthesis also contributes to growth. We have investigated the physiological basis for these photoresponses in the argenteum mutant of pea (*Pisum sativum arg*) which permits physical separation of epidermis from mesophyll without damage. Separated cell layers respond to light by growing, acidifying the apoplast, and undergoing a transient depolarization followed by hyperpolarization of the plasmamembrane. Acidification by epidermal cells is stimulated non-photosynthetically by blue and red light. The response to red, but not blue, is reversed by far red light implying that phytochrome acts separately from a blue-light receptor to stimulate acidification. External barium inhibits acidification in both red and blue light, but the inhibition is reversed by added calcium. Light-induced depolarization of the epidermal cell is dependent on external calcium, and is not inhibited by barium. In contrast, depolarization of the mesophyll cell depends on external chloride, not calcium. A role for light-regulation of ion channels and a proton pump in both cell types is proposed. These distinct responses to light of epidermal versus mesophyll cells normally operate in concert to mediate light-driven expansion of green leaves.

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ION CHANNELS IN THE PLASMAMEMBRANE OF EPIDERMAL AND MESOPHYLL CELLS OF GROWING PEA LEAVES

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Ion currents across the plasmamembrane of mesophyll and epidermal cells have been compared with respect to voltage dependence, kinetics, ion specificity, and sensitivity to cytoplasmic calcium levels. Patch-clamp electrophysiological experiments were conducted both in the whole-cell and isolated-patch configurations. Whole-cell anion currents of epidermal cells are activated by hyperpolarization and do not inactivate with time, while anion currents in mesophyll cells show activation at hyperpolarized voltages and voltage-dependent inactvation in the presence of ATP. Mesophyll anion currents are strongly stimulated by increasing calcium from 0.1 to 1 um. An ion channel in mesophyll cells is described with similar calcium dependence. In epidermal cells, one anion and one cation channel were dominant in most excised patches. The anion channel conducts nitrate, halides and malate, has a conductance of 300 pS in symmetrical 100 mM Cl^-, and can be blocked by SITS from the cytoplasmic side. The cation channel poorly discriminates between K^+, Na^+, and Li^+, has a conductance of 35 pS in symmetrical 100 mM K^+, is not blocked by either TEA or Ba^{++}, and shows increased open probability when Ca^{++} is raised from 0.1 to 1 um on the cytoplasmic side. The properties of these channels suggest their involvement in light-stimulated depolarization of growing leaf cells, and a model for their action will be presented.

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CALCIUM TRANSPORT IN RIGHT-SIDE-OUT PLASMA MEMBRANE VESICLES OF WHEAT ROOTS: CHARACTERIZATION OF VOLTAGE-GATED CALCIUM CHANNELS

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Calcium plays an important role in the regulation of many plant cellular processes. The primary pathway for entry of Ca^{2+} into the plant cell presumably involves plasma membrane Ca^{2+} channels, although to date they have not been well characterized. In this study, we investigated $^{45}Ca^{2+}$ transport in right-side-out plasma membrane vesicles isolated from roots of two wheat cultivars (Al-tolerant Atlas and Al-sensitive Scout) using aqueous polymer two-phase partitioning. Marker enzyme analysis indicated that the plasma membrane preparations were nearly free of contaminating endomembranes, and ATPase latency studies demonstrated that the vesicles were 85-95% right-side-out. The voltage dependence of Ca^{2+} influx was studied by measuring $^{45}Ca^{2+}$ influx in vesicles in response to transmembrane electrical potentials generated via K^+ diffusion potentials, using a K^+-valinomycin clamp. Ca^{2+} influx was found to be strongly voltage-dependent. Maximal Ca^{2+} influx occurred at -100 mV, while no Ca^{2+} influx was observed at E_m values more negative than -170 mV or more positive than -25 mV. Voltage-dependent Ca^{2+} influx over a range of external Ca^{2+} concentrations (10 to 500 μM) was completely blocked by 5 μM La^{3+}. The concentration-dependency for Ca^{2+} influx followed Michaelis-Menten kinetics, with a K_m of approx. 170 μM, and it was observed that this channel was selective for Ca^{2+} over Mg^{2+} and Sr^{2+}. We are currently investigating whether Al^{3+} also blocks this Ca^{2+} channel. Additionally, we are determining whether Al^{3+} preferentially blocks Ca^{2+} channels in plasma membrane vesicles derived from Al-sensitive Scout in comparison with Al-tolerant Atlas, which was suggested from earlier Ca^{2+} transport studies with intact roots from these two cultivars.

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AN INVESTIGATION OF THE KINETICS OF ALUMINUM UPTAKE IN CELL SUSPENSIONS OF *PHASEOLUS VULGARIS* L.

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The kinetics of aluminum (Al) uptake in cell suspensions derived from an Al-tolerant (Dade) and an Al-sensitive (Romano) cultivar of *P. vulgaris* were investigated. With 75 µM $AlCl_3$ in the uptake solution, uptake was rapid during the first 20 minutes with little additional absorption occurring over the remainder of the 180 minute experimental period. In contrast, similar studies with excised roots showed that the rapid phase of uptake occurred over a longer period of time and was followed by a linear phase of uptake. Further studies suggested that the differences in Al uptake between excised roots and cell suspensions were an artifact of our new experimental system rather than a reflection of real differences in uptake between the two systems. The rapid, extensive and saturable nature of uptake in our cell system suggests that depletion of Al from uptake solutions could be responsible for the lack of an observable linear phase of uptake. In further support for this hypothesis, accumulation of Al on a dry weight basis decreased with increasing cell density. However, when the concentration of Al in our uptake solutions was increased to 500 and 1000 µM, total accumulation of Al increased but the general pattern of uptake was not affected. Increasing the concentration of Al in our uptake solutions led to concerns about precipitation of Al interfering with our ability to measure Al accumulated by cell suspensions. Thus, experiments with high volume (50 mL), low concentration (75 µM) absorption solutions were also performed. Upon increasing the volume of uptake solutions a biphasic pattern of uptake was observed, with a rapid phase of uptake during the first 30 minutes, and a linear phase of uptake over the remainder of the 180 minute uptake period. Our studies suggest that it is possible to investigate the kinetics of Al uptake at the cellular level, which in turn could provide us with a means to directly measure the uptake of Al across the plasma membrane.

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CLONING AND CHARACTERIZATION OF A PLANT PROTON-AMINO ACID SYMPORT BY FUNCTIONAL COMPLEMENTATION OF A YEAST AMINO ACID TRANSPORT MUTANT

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Amino acids are transported across the plasma membrane of plant cells by proton-amino acid symports. We report here the successful cloning of a neutral amino acid carrier by functional complementation. A histidine transport deletion mutant of *Saccharomyces cerevisiae* was transformed with an *Arabidopsis* cDNA library constructed in a yeast expression vector. Forty transformants, out of 10^5, allowed growth on a histidine limiting medium. The acquired ability to grow on low histidine was shown to be strictly dependent on the protein encoded by the expression plasmid. Histidine and alanine transport activity were 20-fold greater in the transformants The transport kinetics, inhibitor sensitivity, and substrate specificity closely match those of a carrier we previously described in plasma membrane vesicles isolated from leaf tissue. The cDNA insert is 1.7 kb with an ORF that codes for a protein composed of 486 amino acids. Hydropathy analysis of the deduced amino acid sequence suggests this is an integral membrane protein with 11 to 13 transmembrane alpha-helices. Overall, the sequence of this amino acid carrier is not closely related to any other protein sequences in the Genbank database. Interestingly, however, there are small regions of sequence that exhibit significant levels of similarity with at least six other integral membrane proteins. Further analysis of expression, regulation, and function of this clone should provide new insight into its role in assimilate partitioning and plant cell biology.

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ATP REGULATION OF THE PLASMODESMAL SIZE EXCLUSION LIMIT IN WHEAT ROOTS

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Primary plasmodesmata (PDM) have a size-exclusion limit (SEL) of 800-900 Da; i.e., molecules of molecular weight >SEL cannot diffuse into neighboring cells via the cytoplasmic annulus. An investigation of the SEL of wheat roots was undertaken to answer two questions; is the SEL affected by the ATP levels of cells, and can ATP move across PDM? Epidermal and cortical cells of the root-hair zone of 3-day-old wheat roots were pressure-injected with Lucifer Yellow (mw 457) or 1 to 10 kDa fluorescein-dextrans, and the ability of the dye to move to neighboring cells was determined by fluorescence microscopy. In the absence of metabolic inhibitors, the SEL was <1 kDa. The ATP analog 2'-O-(trinitrophenyl)adenosine-5'-diphosphate (mw 681) moved freely across the PDM. This indicates that ATP and ADP should also be able to equilibrate between cells via the PDM. Upon reduction in the ATP level with 1 or 10 mM azide the SEL increased slowly, and had risen to between 5 and 10 kDa by 1 hr. N_2 caused a similar increase in the SEL. It appears that PDM are constricted by an ATP-dependent process so as to maintain a low SEL limit. When roots are subjected to anaerobic conditions, the increase in the SEL should permit increased influx of sugars into the anaerobic region so as to enhance anaerobic respiration, and perhaps increased influx of ATP from aerobic regions. Supported by grants from NASA to REC and NSF to WJL.

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EFFECTS OF ETHYLENE ON TRACHEID PRODUCTION IN *ABIES BALSAMEA* (L.) MILL. STEMS

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Dormant, 1-year-old balsam fir shoots either were left intact or were debudded and treated apically with lanolin containing 0, 0.1 or 1 mg IAA g^{-1}. These shoots were placed under controlled environment conditions favorable for growth, with their basal end immersed in various concentrations of ethrel, cobalt chloride or silver thiosulfate. Tracheid production and ethylene evolution were measured at weekly intervals during the 4-week culture period. Cambial reactivation was induced by IAA and associated in time with increased ethylene evolution, but the IAA-induced tracheid production was not correlated with the amount of ethylene evolved. In IAA-treated cuttings collected at different times during the transition between the dormancy stages of rest and quiescence, ethylene production was increased by ethrel and decreased by cobalt ions, whereas silver ions had no effect. Tracheid production was unaffected by any treatment, except for the highest cobalt and ethrel concentrations, which were inhibitory. In a second experiment performed in a greenhouse with intact 2-year-old seedlings, ethrel applied in lanolin near the base of the previous year's leader stimulated tracheid production at the application point but not 5 cm above. However, ethylene production was markedly increased at both positions. The results suggest that IAA-induced tracheid production is not mediated through ethylene.

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POLLEN TUBE GROWTH IS NOT SUFFICIENT TO INDUCE ETHYLENE BIOSYNTHESIS AND SENESCENCE IN CARNATIONS

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Pollination in many flowers serves as an external signal for the initiation of petal senescence. In carnation flowers, pollination has been correlated with an increase in stylar ethylene that in turn leads to the induction of ethylene production and senescence of the petals. Two carnation pollen types, one which induces stylar ethylene and petal senescence ('Starlight') and one which does not (C), have been studied in an attempt to understand the pollen-pistil interactions which lead to increased ethylene biosynthesis. Both pollen types share similar morphologies, similar growth rates of pollen tubes through the length of the style, and similar ACC contents. In contrast, only 'Starlight' pollen is capable of successfully fertilizing the ovary, inducing petal senescence, and inducing stylar ethylene. Stylar ethylene induced by 'Starlight' pollination is characterized by two peaks- one which begins at 6 hours and peaks at 12 hours and one that begins after 24 hours. Northern blots of total RNA from either Starlight pollinated or C pollinated styles were probed with a pollination inducible ACC synthase from carnation (CARAS1). Pollination with 'Starlight' leads to increased levels of this transcript. CARAS1 first appears 6 hours post-pollination, correlating with the induction of the first peak of stylar ethylene, and remains elevated in the style through 48 hours post-pollination. In contrast, the senescence related ACC synthase (CARACC3) shows no induction in pollinated styles. This suggests that in order to have successful fertilization and induction of senescence, the pollen-pistil interaction must induce ACC synthase expression and ethylene biosynthesis in the style. In addition, this suggests that growth of the pollen tube alone is not sufficient for elicitation of this response.

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DELETION OF THE COOH-TERMINUS RESULTS IN A MORE ACTIVE, MONOMERIC 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE

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ACC synthase is a key enzyme in plant ethylene biosynthesis. We have isolated a wound-inducible ACC synthase cDNA (pTACC-B1) from tomato (*Lycopersicon esculentum* cv Pik-Red) and expressed it as a functional enzyme in *Escherichia coli*. Using this bacterial expression system, we have tested the role of COOH-terminus in affecting the conformation and activity of a single isoform of ACC synthase. A nest of deletion mutants of ACC synthase was created, which were expressed in *E. coli* and the proteins purified and tested for activity, kinetic parameters and conformation. Deletion of the C-terminus including Arg^{429} resulted in a totally inactivated enzyme. Deletion of 46 to 52 amino acids from the C-terminus generated an ACC synthase which fractionated on gel filtration as a monomer and had higher affinity for the substrate S-adenosylmethionine compared to the wild-type enzyme, which appeared to be a dimer. These results demonstrate that the C-terminus of ACC synthase not only affects its activity but also influences its dimerization.

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CO_2 AND EFE ACTIVITY IN ROOTS OF SUNFLOWER SEEDLINGS.

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Ethylene has been implicated in controlling the growth and development of many plant tissues, including roots. ACC (1-aminocyclopropane-1-carboxylic acid) is the immediate biosynthetic precursor of ethylene in higher plants, and is converted to ethylene in an oxidation reaction by the enzyme EFE (also known as ACC oxidase). Recently a method has been developed to extract and assay authentic EFE activity *in vitro* (Ververidis and John, 1991). The enzyme requires reduced iron, ascorbate and CO_2 to function and the activity has been shown to be enhanced greatly by high levels of exogenous CO_2 (Dong et al., 1992). EFE was extracted from aeroponically grown roots of sunflower seedings, using an anaerobic environment and reduced iron and ascorbate in the extraction buffer. The activity of the enzyme was assayed under aerobic conditions with and without high levels of exogenous CO_2, varying the parameters of reduced iron, ascorbate, pH, temperature and substrate. It was found that CO_2 had no effect on the optimal temperature of the enzyme. High CO_2 decreased the optimal reduced iron concentration, and increased the optimal ascorbate level. CO_2 radically increased the V_{max} of the enzyme but reduced the K_m. The pH optimum of the enzyme was shifted lower with high CO_2. The possibility that CO_2 may have a major influence on ethylene production by tissues *in vivo* will be discussed.

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CYTOKININS AND GRAIN DEVELOPMENT IN MAIZE

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The results of many previous studies have implicated cytokinins as having a role in cereal grain development, but the exact nature of that role has remained obscure. This study has been undertaken to investigate the function of cytokinins in developing maize grains. Cytokinins extracted from maize kernels harvested from 1-10 days after pollination (DAP) were isolated by immunoaffinity chromatography (IAC), separated by HPLC, and quantitated by radioimmunoassay (RIA). The activity of cytokinin oxidase, a cytokinin degrading enzyme, was determined in an assay by TLC. In order to determine the effect of increased cytokinins within maize plants a stem injection technique was used to introduce known quantities of cytokinins into the stems of field-grown plants at the time of pollination. The primary cytokinins found within the developing kernels were zeatin (Z) and zeatin riboside (ZR). Both reached their peak at 9 DAP. At its peak, the level of ZR increased up to 10-fold over the basal level (from 5.5 ng/gFW to 58.4 ng/GFW), while the amount of Z within the kernel tissue peaked at a level 5-fold over the basal level (increasing from 4.4 ng/GFW to 22.3 ng/GFW). Cytokinin oxidase levels showed a slight increase from 3-6 DAP then increased 4-fold through 10 DAP. Cytokinin stem injections into maize plants induced a yield increase of up to 30% over control injections, with the primary increase a result of an increased kernel number rather than an increase in kernel weight. This study confirms the presence of a transient increase in cytokinins within developing maize kernels as has been found in young rice and wheat grains. The activity of cytokinin oxidase within the kernels increased as Z and ZR levels rose and was still increasing as the levels of the cytokinins had begun to decline. Increasing the cytokinin complement within maize plants appears to have lowered the kernel abortion rate and allowed more kernels to continue on to maturity, which produced a significant increase in yield.

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HIGH ENDOGENOUS ISOPENTYL ADENINE CONTENT COINCIDED WITH AN EXTREMELY SHOOTY RICE PHENOTYPE

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We have characterized from anther culture of homologous tetraploid rice an extremely shooty mutant which could produce 150-200 buds from a single shoot within a month (Qin, R-z. et. al.,1989, Acta Botanica sinica, 31:830-834). When these buds were chopped into small pieces and cultured on MS differentiation medium, shooty phenotype was maintained from about 50% of the calli. This characteristic is inherited by subcultures of all the generations to date without loss of the vigorous multiplication frequency. Mature and fertile plants can be produced by individual shoot under higher temperature and longer photoperiod (28-30°C, 10-12 hr light for plant regeneration compared to 20°C, 8-10 hr light for tissue culture). Examination of major known endogenous plant hormones by HPLC and GC-MS has revealed that the mutant had about 5-10 times higher 2IP content than that of the parental type while maintained more or less the same IAA, GA and ABA levels. Shooty phenotype can be partially reproduced from wild-type rice culture using MS medium supplemented with elevated IP level. We are now trying to clone cDNA sequence(s) responsible for this phenomenon by differential hybridization.

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ISOLATION AND CHARACTERIZATION OF A *CIS-TRANS* ISOMERASE OF ZEATIN

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Conversion of *cis*-[^{14}C]zeatin to *trans*-[^{14}C]zeatin in immature seed of *Phaseolus vulgaris* led to the isolation of an isomerase from the endosperm. The isomerase was purified 2000 fold by anion exchange (Mono Q), gel filtration (Superose 12), hydophobic interaction (Phenyl Superose), and Concanavalin A chromatography. Retention of the enzyme on the Concanavalin A column indicated that the enzyme is a glycoprotein. Although the isomerase mediates conversion in both directions, formation of *trans*-zeatin is favored. The ribosides of *cis*- and *trans*-zeatin were also found to be substrates for the enzyme. Light and flavin were necessary for the conversion. Both FMN and FAD were effective cofactors. DTT was required for the reaction, with the optimal concentration decreasing with increasing purity of the enzyme. The enzyme could be stored at -80°C for at least 8 weeks without loss of activity. The occurrence of a *cis-trans* isomerase indicates that *cis*-zeatin and its riboside resulting from tRNA turnover can serve as a source of biologically active cytokinins.

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LOCALIZATION AND CLONING OF A CYTOKININ METABOLIC ENZYME, ZEATIN O-XYLOSYLTRANSFERASE

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In immature seeds of *Phaseolus*, zeatin is rapidly converted to O-xylosylzeatin or O-glucosylzeatin, depending on the species. The enzymes mediating these conversions have been partially purified and monoclonal antibodies specific to these enzymes were produced. Tissue printing and cytolocalization indicated that the enzyme is present in both the nuclei and the cytoplasm of the endosperm. Further analyses of the nuclear fractions showed that the protein is associated with the nuclear pellet, suggesting that the enzyme may have a dual function. The monoclonal antibody has been used to identify immuno-positive clones in a λgt22 expression library. Sequence analysis of the positive clones revealed an open reading frame encoding a 51 kD protein which is in agreement with the mass of the enzyme estimated by SDS-PAGE. *In vitro* expression systems are being employed for enzyme activity analyses.

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IMPACT OF WATER DEFICIT ON THE DISTRIBUTION OF IAA, ABA, ACC, AND ETHYLENE IN ELONGATING LEAVES OF *ZEA MAYS*

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Plant chemicals elicited during periods of water deficit are involved in the signaling mechanism that inhibits plant growth. Abscisic acid (ABA) is considered to be one of these inhibitory compounds. The roles of indole-3-acetic acid (IAA) and ethylene in the growth-limiting process triggered by water deficit are not as clear. To gain additional insight into their respective roles, the levels of IAA, IAA conjugates, ABA, 1-aminocyclopropane-1-carboxylic acid (ACC), and ethylene were measured in the expanding basal section and expanded apical portion of leaves from water-stressed and nonstressed greenhouse-grown *Zea mays* plants. Despite sufficient water deficit to reduce tissue water potential and growth, the tissue-specific levels of IAA and IAA conjugates remained unchanged throughout the leaf. ABA increased to equal levels in both the elongating and expanded segments of leaves from stressed plants. Ethylene production and ACC were detected only in tissue from the basal, elongating portion of stressed leaves. However, ethylene production decreased by 75% in the expanding, basal leaf tissue sampled during the afternoon from stressed plants. ACC levels in this tissue remained unchanged. Pools of the same phytohormones and metabolites were determined in the root systems from stressed and nonstressed plants. The implications of these observations will be discussed relative to decreases in growth during water deficit.

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PHYTOHORMONE REGULATION OF A 3-HYDROXY-3-METHYLGLUTARYL-COENZYME A REDUCTASE (HMGR) GENE FROM *Camptotheca acuminata.*

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C. acuminata is a tree species that produces the anticancer monoterpenoid indole alkaloid camptothecin (CPT). HMGR is a branch-point enzyme which shunts HMG-CoA into the isoprenoid pathway, and supplies mevalonate for the synthesis of the terpenoid component of CPT. Recent studies in our group have shown that the expression of a *Camptotheca* HMGR gene (*hmg1*) is differentially regulated by wounding and methyl jasmonate. Regulation of the *hmg1* promoter was studied in transgenic tobacco using three translational fusions (-1678, -1107, -165) with the ß-glucuronidase (GUS) reporter gene. In the present study, we examined the effect of different phytohormones on the expression of these *hmg1*::GUS constructs. Leaf disks (7 mm) and whole leaves were treated with 10^{-6}, 10^{-5},or 10^{-4} M solutions of ethephon, ABA, IAA or kinetin and assayed for GUS activity over 72 hr. Ethephon, ABA and IAA had no effect on *hmg1*::GUS expression during the 72 hr treatment. In contrast, kinetin treatment stimulated a concentration dependent increase in GUS expression in all three *hmg1*::GUS constructs. These results suggest that the regulation of this gene is insensitive to ABA levels but responds positively to cytokinins, which is unexpected in view of the importance of mevalonate in the biosynthesis of both phytohormones.

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THE *IMMUTANS* VARIEGATION MUTANT OF A. THALIANA

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The *immutans* variegation mutant of Arabidopsis thaliana (Columbia background) displays a pattern of white and green sectoring that is influenced by light intensity and/or temperature, with higher levels of either eliciting increased white tissue sectors. Observation by electron microscopy and fluorescence microscopy revealed that cells in white tissue primarily contain non-functional colorless plastids with only an occasional normal green chloroplast present, while green tissue is phenotypically the same as wild-type. Genetic analysis showed that *immutans* is a nuclear recessive gene that maps to chromosome 4, and that white and green cells have a uniform genetic constitution. The nature of the mutation appears to be distinct from that of position effect variegation, transposon activity, or organelle mutators that have been described. Pigment analysis of white versus green tissue revealed that white cells accumulate the colorless carotenoid precursor, phytoene. However, the mutation does not genetically map to the same location as the gene for phytoene desaturase, the enzyme responsible for the conversion of phytoene into zeta-carotene in the chloroplast-localized carotenoid biosynthetic pathway. White tissues display decreased mRNA levels of both chloroplast- and nuclear-encoded genes for chloroplast proteins, suggesting that the hypothesized "chloroplast signal" has been affected as a result of the mutation. Models will be presented for possible functions of the Immutans gene product and how it may affect phytoene desaturase, along with results from both biochemical and molecular genetic analyses of *immutans* plants.

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CHARACTERIZATION OF GLUTATHIONE S-TRANSFERASE FROM *ARABIDOPSIS THALIANA*

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Glutathione S-transferases (GST) are involved in cellular detoxification by conjugating GSH to a variety of electrophilic compounds. We have isolated a GST cDNA (GST2) from *Arabidopsis* and shown that the expression of this gene is regulated by ethylene. GST2 mRNA is induced after exposure to ethylene for two hours and remains inducible throughout the life of the plant. Other stimuli also increase the abundance of GST2 mRNA, including mechanical stress, wounding and salicylic acid (SA). Mechanical stress and SA also induce GST2 mRNA in the ethylene-insensitive mutant (ETR-1) indicating that ethylene is not the only modulator of GST2 expression. Two closely related genomic sequences have been isolated. One encodes the GST2 mRNA while the other contains a pseudogene. Expression of GST2 cDNA in *E. coli* yields a 25 Kd protein, as predicted from the cDNA sequence. This protein can be purified by GSH-affinity chromatogrphy and has GST activity using chloro-2,4-dinitrobenzene as substrate. Proteins of similar molecular weight have been purified from *Arabidopsis* leaves and also shown to have GST activity.

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BINDING OF A 50-KD PROTEIN TO THE U-RICH DOMAIN IN THE 3'-UTR OF ELICITOR DOWN-REGULATED BEAN *PvPRP1* mRNA

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The *PvPRP1* mRNA is known to be destabilized in bean (*Phaseolus vulgaris*) cells after fungal elicitor treatment (Zhang et al., submitted). To investigate the mechanism of the destabilization, ^{32}P-labeled *PvPRP1* RNA was incubated with cellular protein extract, and interacting protein was detected by UV cross-linking and gel retardation assay. A 50-KD protein (designated PRP-BP) was identified which binds specifically to the 3'-partion of the *PvPRP1* RNA. The binding site was localized to a 27 nt U-rich (60%) sequence in the 3'-untranslated region by using RNAs transcribed from Exo-III nested deletion clones. Competition experiment showed that the cold poly-U competes for the PRP-BP binding activity, although much less effectively compare to RNA with the PRP-BP binding site. The PRP-BP activity is optimum at pH7.0. Metal ions, such as K^+, Mg^{2+} and Ca^{2+} inhibit PRP-BP activity. Reducing agents, DTT and β-ME strongly stimulate the binding activity. In elicitor-treated cells, PRP-BP activity increases about 5-fold prior to the fast *PvPRP1* mRNA degradation, suggesting that this RNA binding protein might be involved in the destabilization of *PvPRP1* mRNA in elicited bean cells.

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LENGTH HETEROGENEITY IN THE 3' UNTRANSLATED REGIONS OF RUBISCO SMALL SUBUNIT MESSENGER RNAs IN SUGARCANE

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Sugarcane (*Saccharum* hybrid variety H32-8560), a polyploid, is estimated to contain about 16 copies of the Rubisco small subunit (rbcs) gene. A full length rbcs gene (scrbcs-1) was isolated from a sugarcane genomic library and sequenced. Using oligo(dT) and a rbcs-specific oligomer (within the coding region) as primers, the Rapid Amplification of cDNA 3' Ends (3'-RACE) technique was used to clone the rbcs mRNA 3' regions from different sugarcane tissues, including green callus (actually leaf primordia), very young leaf, young leaf, mature leaf, leaf sheath, and greening leaf. The sequences of the 3'-RACE products, unlike many other rbcs gene members which are heterogeneous in their 3' untranslated regions, are all very similar to the 3' end of scrbcs-1. However, their lengths are quite different, and can be classified into five different size groups (excluding the polyA tail), i.e. Group I to V, 500bp, 350bp, 250bp, 180bp and 140bp, respectively. Northern analysis of total RNA from mature leaf, as well as sequence determination of the rbcs cDNA clones isolated from mature leaf cDNA library, confirms the existence of different lengths of rbcs mRNA. Polymerase Chain Reaction analysis of sugarcane genomic DNA suggests that the genes encoding Group I and II mRNAs have the same length. The ratio of these two classes of mRNAs in various sugarcane tissues is similar. The significance of this length heterogeneity will be discussed.

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SEQUENCES INVOLVED IN RAPID SAUR MRNA TURNOVER

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The SAUR genes from soybean are very strongly auxin-induced. Their expression is also correlated with tropistic responses of the plant in which auxin redistribution occurs. Tissue print analysis of horizontally oriented soybeans has suggested a very short half-life of this mRNA in the elongating hypocotyl region, consistent with the time course of the tropistic response. To examine the sequences responsible for the short half-life of the SAUR mRNA we constructed transgenic tobacco plants containing various chimeric SAUR genes fused to several different promoters, 3' noncoding sequences, and coding sequences. We have found that the coding sequence of the SAUR mRNA from soybean is sufficient for mRNA accumulation induced by the translational inhibitor cycloheximide. We have further shown that the cycloheximide-induced block on translation results in an increase in the half-life of the SAUR mRNA. Using a heat shock-inducible promoter fused to the SAUR coding region, we were able to measure the half life of this chimeric mRNA as 27 minutes in the absence of cycloheximide, and 84 minutes in the presence of cycloheximide. We examined the requirement for translation of the mRNA for its rapid turnover, as well as the role of flanking noncoding sequences in other aspects of SAUR rapid turnover not disrupted by cycloheximide. We postulate the existence of a site-specific instability factor in plant cells which either degrades the SAUR mRNA itself or targets them for rapid degradation. The relationship of this rapid turnover to gene regulation in growth and tropistic responses is discussed.

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WIN4, A FAMILY OF WOUND-INDUCED GENES IN POPLAR TREES

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Two *wound-in*ducible cDNAs (*win*4) from poplar leaves show sequence similarity (75% identity at the nucleotide level) to bark storage proteins (BSP) that accumulate seasonally in poplar bark tissues. Based on these data, our working hypothesis is that *win*4 genes encode leaf vegetative storage proteins in poplar trees. *win*4 genes are locally and systemically wound-inducible in leaves, are expressed after exposure of leaves to airborne methyl jasmonate, but are not expressed in leaves or stems under short days.

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AUXIN PHYSIOLOGY AND EXPRESSION OF THE GH3 PROMOTER-GUS FUSION GENE

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The GH3 gene from soybean is specifically and rapidly induced by auxin. The expression of a GH3 promoter-GUS fusion gene (GH3-GUS) in transgenic tobacco plants is not normally detectable in vegetative organs, but can be induced by exogenous auxin in every cell in a dose-dependent manner, indicating that a critical auxin concentration is a limiting factor for the fusion gene activation. Although GH3 exhibits a completely different pattern of expression as compared to that of SAURs, which are also auxin-inducible, the expression of GH3-GUS in transgenic tobacco also responds to gravity as SAUR promoter-GUS fusion genes do. The activation of GH3-GUS by exogenous auxin applied to decapitated apices can be influenced by gravity and unilateral light with significantly higher promoter activities in the bottom side (gravistimulated) or in the dark side (unilateral light-stimulated). The pattern of ^{14}C-IAA transport/distribution matches well with the patterns of the activation of GH3-GUS in transgenic plants under gravistimulation. In the basal ends of cut stems where adventitious roots normally initiate, the activation of GH3-GUS is also observed several hours after cutting. In addition, the activation of GH3-GUS has been detected in a single interfascicular parenchyma cell in stems where adventitious roots are initiating, possibly indicating the onset of root initiation. In undecapitated plants, the expression of GH3-GUS is also detectable in a small number of cells in nodes, indicating that the expression of the fusion gene may be a useful tool to study the involvement of auxin in apical dominance. Since the expression of GH3-GUS can be induced to a high level with exogenous auxin in all cell types, the activation of GH3-GUS is likely due to elevated levels of endogenous auxin within these cells and tissues. We are currently testing whether the expression of GH3-GUS can be used as a molecular assay system to monitor changes in auxin concentrations *in planta*.

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PROMOTER ANALYSIS OF AN ETHYLENE-INDUCIBLE BEAN ABSCISSION CELLULASE GENE

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Ethylene-induced bean leaf abscission correlates with the *de novo* accumulation of pI 9.5 cellulase in the abscission zone. A bean genomic library was screened with a cDNA (pBAC10) encoding the pI 9.5 cellulase. Two overlapping genomic clones were identified which together encode the entire bean abscission cellulase (BAC) coding region and up to 5 kbp of 5' flanking sequence. Results from Southern blot hybridization are consistent with there being a single copy of a gene representing pBAC10 in bean. Ethylene-inducible and tissue-specific expression of a series of BAC promoter deletion constructs fused to the coding region of the β-glucuronidase (GUS) reporter gene was analyzed following *Agrobacterium*-mediated transformation of tomato. Tomato was chosen because it is easily transformed and regenerated, and leaf abscission in tomato is ethylene-inducible and correlated to an increase in abscission zone cellulase activity. A promoter construct containing 210 bp of sequence 5' to the transcription start site was found to be sufficient to drive low levels of GUS expression in tomato abscission zones as early as 24 hours following ethylene induction. Expression in ethylene-induced abscission zones was several fold higher than in adjacent petiole tissue.

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SOYBEAN G-BOX BINDING FACTORS: THE IDENTIFICATION OF A PUTATIVE NEGATIVE REGULATOR ON TRANSCRIPTION ACTIVATION BY GBFS

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The G-box is a cis-acting element found within the promotor of many plant gene which is characterized by a palindromic DNA motif of GCCACGTGGC. Gel retardation analysis using the G-box motif as a probe indicated that soybean has multiple G-box binding factors (GBF). Here we report the isolation of three soybean cDNA clones encoding GBF proteins referred to as SGBF-1, SGBF-2a, and SGBF-2b. The deduced amino acid sequence of three cDNAs indicate that each encodes a protein carrying basic leucine zipper (bZIP) structure as a DNA binding domain. The SGBF-1 and SGBF-2a are similar to other plant GBFs as characterized by an N-terminal proline-rich domain. Two forms of SGBF-2 are identified in soybean. SGBF-2a is the larger RNA encoding 424 amino acid protein, whereas the SGBF-2b is the smaller RNA resulting from the removal of 178 nucleotides from SGBF-2a RNA. The resulting protein encode 366 amino acid protein that lack 58 amino acid at N-terminal region. This deleted region is equivalent to the N-terminal proline-rich domain (20% Pro) of Arabidopsis GBF-1 that was shown to activate the transcription in both plant and mammalian cells. This indicate that SGBF-2b is a trans-negative regulator similar to the mammalian bZIP factor FosB2 (Yen et al., 1991. Proc. Natl. Acad. Sci. U.S.A. 88, 5077-5081.). This suggest that plant has negative regulator to GBFs which will generate additional diversity for gene regulation by protein interaction from multiple GBFs.

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REGULATION OF *DE NOVO* PURINE BIOSYNTHESIS IN UREIDE-PRODUCING LEGUMES

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In tropical legume root nodules, ureides are synthesized via oxidation of *de novo* made purines. To study regulation of purine biosynthesis, we isolated cDNA clones encoding aminoimidazole ribonucleotide (AIR) carboxylase and aminoimidazole-4-N-succinocarboxamide (SAICAR) synthetase from *Vigna aconitifolia* library by functional complementation of *purE* and *purC* mutations in *Escherichia coli*. We also isolated cDNA clones encoding phosphoribosylamido transferase (*purF* gene product) which apparently regulates this pathway. Sequence analysis of the *purC* and *purE* cDNA clones revealed that the *purE* gene is fused with *purK* as is the case in yeast. This differs from that in humans where ade2 gene represents a fusion of the *purC* and *purE*. The aminoterminus of *Vigna* SAICAR synthetase protein, which resembles the *purK* gene product, is not required for complementing *purE* mutation. Soybean *purF* cDNA contain long 5' region, and its deduced amino acid sequence shows homology to other proteins destined for plastids in plants. Both soybean and mothbean clones have three conserved amino acids of 11-amino acid propeptide, found in *Bacillus subtilis* and chicken, in its 5' region downstream of targeting sequence, and also contain [Fe-S] motif at their C-terminal region. Mothbean cDNA, even though lacking 5'-end ATG sequence, complemented *E. coli purF*. Primer extension study showed the expression of *V. aconitifolia* PRPP-AT mRNA in early nodules and its increase as the nodules matured. Furthermore, the expression of *purF* is higher than and preceeds that of *purC* gene. These genes are induced in root nodules following commencement of nitrogen fixation and their expression is not detected in roots.

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ISOLATION AND CHARACTERIZATION OF cDNAS ENCODING 1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE HOMOLOGS FROM MUNG BEAN HYPOCOTYLS

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By screening a mung bean (Vigna radiata L.) hypocotyl cDNA library using the combination of apple (pAE12) and tomato (pTOM13) ACC oxidase cDNAs as probes, cDNA clones encoding ACC oxidase homologs were isolated. Based on restriction enzyme map and DNA sequencing analyses, they can be divided into two homology classes, represented by pMBAO1 and pMBAO4. While pMBAO1 and pMBAO4 exhibit close homology at their coding segments, their 3'-noncoding regions are divergent due to numerous insertions/deletions and substitutions of nucleotides. pMBAO1 is 1312 bp in length and contains a single open reading frame encoding 317 amino acids (M.W.=35.8 kD), while pMBAO4 is 1172 bp long and is a partial cDNA clone encoding 308 amino acids. Their deduced amino acid sequences share 83.4% identity, and display sequence conservation (73-86%) to other ACC oxidases from various plant species. Northern blot analysis of RNAs isolated from hypocotyl, leaf, and stem tissues using gene specific probes generated by polymerase chain reaction showed that pMBAO1 transcript is detected in all parts of the seedling and that the level in hypocotyls is further increased following excision. The time course of changes in the level of ACC oxidase transcript and enzyme activity following excision was investigated. The maximum induction of ACC oxidase transcript occurred at 6 h after excision, while the maximum enzyme activity was observed at 24 h, indicating that wound-induced ACC oxidase gene expression may be subject to both transcriptional and posttranscriptional control.

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A MULTI-DOMAIN CYSTATIN FROM POTATO

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The protein crystals found in potato tuber skin cells are comprised of a single ~85-kD polypeptide that is a potent inhibitor of papain and other cysteine proteases (Rodis & Hoff, Plant Physiol. 74: 907). We have characterized this inhibitor in more detail. Titrations of papain with the inhibitor show that it can bind eight papain molecules simultaneously with Ki = 0.1 nM. Treatment of the inhibitor with trypsin and other non-specific proteases cleaves the polypeptide into one 32-kD and five 10-kD fragments which all retain the ability to potently inhibit papain. Partial amino acid sequencing of these polypeptides show that they are all related to the cystatin superfamily of protease inhibitors. We have characterized a complete genomic copy of the inhibitor gene. It encodes a polypeptide of 86,778 D which is comprised of eight tandem cystatin domains. The domains are 63-93% homologous, and have variations in amino acid sequence in the regions involved in protease binding. We propose the name potato multicystatin for this unique cysteine protease inhibitor. The multicystatin is wound-inducible in leaves where it can accumulate to levels of 1-2% total protein. Potato multicystatin therefore adds cysteine protease inhibition to the range of defensive protein activities from potato that may provide pathogen and herbivore protection.

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IN VIVO LABELING OF TOBACCO CELLS WITH ^{14}C-MEVALONATE REVEALS ISOPRENYLATED PROTEINS

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Mevalonate is a precursor of isoprenoid compounds which include, sterols, dolichols, and tRNA's as well as several plant hormones and pigments. Recently, it has been shown that the post-translational, covalent attachment of isoprenoids to proteins may provide a mechanism for membrane association of these proteins. Our objective was to initiate the study of this process in higher plants by identifying isoprenylated protein species and the nature of the isoprenoid adducts. The inhibitor mevinolin (lovastatin) prevents the endogenous production of mevalonic acid through the inhibition of HMG-CoA reductase. The treatment of tobacco cells with low levels of this inhibitor blocks cell growth. Growth can be recovered by the addition of exogenous mevalonate. In this report, it is shown that cultured tobacco cells, when pretreated with mevinolin, incorporate radioactivity from [^{14}C]-mevalonic acid into high molecular mass components. These components are TCA-precipitable, acetone insoluble, RNase resistant, and trypsin sensitive; consistent with the identification of these as isoprenylated proteins. Most of these proteins are membrane-associated and many are similar in mass to *ras*-like GTP-binding proteins and nuclear lamins. We are presently identifying the nature of the isoprenoid groups that modify these proteins by cleavage of the putative cysteinyl thioether bonds with CH_3I and the separation of the $CHCl_3$ soluble components (isoprenoids) by reversed phase HPLC. This work represents direct evidence of protein isoprenylation in higher plants and provides a basis for future work on the role of protein isoprenylation in plant cell growth, signal transduction, and membrane biogenesis.

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IN VITRO ASSAY FOR FARNESYL:PROTEIN TRANSFERASE AND GERANYLGERANYL:PROTEIN TRANSFERASE TYPE I ACTIVITY IN CULTURED TOBACCO CELLS

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Various mammalian and yeast proteins, including small ras-like GTP-binding proteins, heterotrimeric G protein gamma subunits, and nuclear lamins, are covalently linked to isoprenoid derivatives of mevalonic acid (i.e., farnesyl or geranylgeranyl). Isoprenylation of these proteins is required for their assembly into membranes and, hence, for their biological activity. In this report, it is shown that tobacco cell extracts catalyse the transfer of radioactivity from [^{3}H]-farnesyl pyrophosphate and [^{3}H]-geranylgeranyl pyrophosphate to protein substrates *in vitro*. These studies indicate the presence of at least two distinct prenyl:protein transferases in tobacco extracts, one that utilizes farnesyl pyrophosphate and preferentially modifies a substrate protein with a CAIM carboxyterminus (farnesyl:protein transferase), and one that utilizes geranylgeranyl pyrophosphate and preferentially modifies a substrate protein with a CAIL carboxyterminus (geranylgeranyl:protein transferase type I). This work provides a basis for future work on the role of protein isoprenylation in plant cell growth, signal transduction, and membrane biogenesis.

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EVIDENCE FOR THE CONCERTED INVOLVEMENT OF LIGHT, ALKALINE pH AND CALCIUM IN THE C_4 PHOSPHO*ENOL*PYRUVATE CARBOXYLASE (PEPC) KINASE SIGNAL-TRANSDUCTION CHAIN

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Regulation of the light-dependent phosphorylation of C_4 PEPC was studied in isolated mesophyll cells and protoplasts of crabgrass. Due to their open plasmodesmata, C_4-mesophyll cells offer distinct advantages over protoplasts in the study of the PEPC-kinase signal-transduction chain. The apparent phosphorylation status of PEPC was assessed by the target enzyme's malate sensitivity (2.5 mM PEP, ±0.5 mM L-malate, pH 7.3). Malate inhibition of PEPC from leaf and cell extracts was 69 and 63%, respectively, from dark-adapted plants (dephospho-PEPC) and 45 and 30%, respectively, from light-adapted tissue (phospho-PEPC). NH_4Cl, which causes cytosolic alkalization and *in situ* activation of PEPC-kinase in illuminated sorghum mesophyll protoplasts [EJB *210* (1992) 531], decreased the malate inhibition of crabgrass PEPC in illuminated protoplasts and cells 23 and 5%, respectively, suggesting PEPC-kinase was activated in protoplasts but not cells. Cells (from dark-adapted leaves) incubated in media at pH 8.0 + 5 mM $CaCl_2$, in high light, showed a decrease in malate inhibition of PEPC from 63% to 30% (presumably due to *in situ* phosphorylation of PEPC) as compared to cells incubated at pH 7.0 -$CaCl_2$, in the dark, in which malate inhibition remained constant. When only one or two of the three conditions (pH 8.0, 5 mM $CaCl_2$, high light) were present, only a small decrease in PEPC malate inhibition was observed (3-8%). Placing cells from light-adapted leaves in media at pH 7.0 -$CaCl_2$, in the dark, did *not* cause an increase in malate inhibition. These results support the findings of a recent study [EJB *210* (1992) 531] in which changes in Ca^{2+} and H^+ levels were implicated as additional components in the C_4 PEPC-kinase signal-transduction chain.

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UTILITY OF HISTIDINE-TAGGED UBIQUITIN IN THE PURIFICATION OF UBIQUITIN-PROTEIN CONJUGATES AND AS AN AFFINITY LIGAND FOR THE PURIFICATION OF A UBIQUITIN-PROTEIN HYDROLASE

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In eukaryotic organisms, a multienzymatic system catalyzes the covalent ligation of ubiquitin (ub), a 76 aa protein, to intracellular proteins. Specifically, an isopeptide bond is formed between the carboxyl-terminal glycine of ub and the ε-amino group of lysine residues of substrate proteins. Single or multiple lysine residues may be ubiquitinated. Additional ub molecules may also be added by ligation to ub itself at lysine 48, forming polyubiquitin chains. Polyubiquitination is thought to target proteins for degradation. Only a few substrates of the ubiquitin pathway have been identified, however. Efforts to identify new substrates would presumably be enhanced by an approach which permitted the specific purification of ubiquitinated proteins from other intracellular proteins. We report here on the use of a polyhistidine-tagged ub molecule (hisub) both for the purification of hisub-protein conjugates by metal-chelate chromatography and as an affinity ligand for the purification of a ub-protein hydrolase. When *E. coli* extracts containing expressed hisub were passed through a nitrilotriacetic acid-agarose column containing immobilized Ni^{2+} ions (NTA-Ni^{2+} column), hisub was retained. A pH 4.5 wash was used to elute highly purified hisub. Purified hisub and native ub were tested for their ability to form Ni^{2+}-binding ub-protein conjugates in a wheat germ *in vitro* conjugation reaction. In some experiments, wheat germ extracts were preincubated with iodoacetamide to inhibit ub activating and conjugating enzymes. Only those conjugation assays which included hisub and an ATP-regenerating system (without iodoactamide pretreatment) produced a significant level of Ni^{2+}-binding ub-protein conjugates. We also examined the potential of hisub as an affinity ligand for the purification of higher plant ub-protein hydrolases. As a test, a crude lysate of *E. coli* expressing a yeast hydrolase was passed through a hisub affinity column containing hisub bound to NTA-Ni^{2+}-agarose. When the column was equilibrated with buffer containing native ub, the hydrolase was specifically eluted. The expression of hisub in transgenic organisms may provide a useful tool for the specific purification of *in vivo* substrates of the ub-mediated pathway. We are currently testing this possibilty.

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CHANGE IN PEPTIDASE(S) ACTIVITY OF AXES DURING FIRST 24 HR OF COTTON SEED GERMINATION

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Seeds of cotton (Gossypium hirsutum L. M-8, a double haploid) were imbibed in moistened paper towels under standard germination test conditions at 30° C. Axes were excised by dissection from seeds at 0, 3, 6, 9, 12, 18 and 24 hr of imbibition. Separate samples of axes were used for measuring length, fresh and dry weight and peptidase enzyme activity. Tissue was homogenized in 50 mM Tris buffer, pH 7.4, and clarified brei was analyzed for aminopeptidase activity followed by dialysis overnight at pH 5.0 before measuring endopeptidase and carboxypeptidase activities. Significant changes in axial length (4.6 to 10.5 mm) and dry wt (3.5 to 3.2 mg) were observed between 9 and 24 hr of imbibition. Radicle emergence was at 12 hr. Endopeptidase and aminopeptidase activity reached peaks at 12 and 18 hr, respectively. Following a gradual increase in activity between 0 and 18 hr imbibition, carboxypeptidase increased dramatically between 18 and 24 hr. These data indicate that during the first 9 hr of imbibition there is limited axis elongation or degradation of stored reserves by peptidases. This is followed by rapid elongation growth in parallel with reduction in dry wt and marked changes in peptidase activity.

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PURIFICATION AND CHARACTERIZATION OF A PUTATIVE PHOSPHOTYROSINE PHOSPHATASE FROM POTATO TUBER

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A potato tuber acid phosphatase (APase) has been purified 2,289-fold to homogeneity and a final P-Tyr hydrolysing specific activity of 1,936 (μmol Pi produced.min)$^{-1}$/mg protein. Purification steps included: PEG fractionation, S-Sepharose, phosphocellulose, and Phenyl Superose FPLC. Nondenaturing PAGE of the final preparation resulted in a single protein staining band which co-migrated with APase activity. Following SDS-PAGE two protein staining bands of 57 and 55 kD were observed. Western blotting experiments revealed that the 55 kD subunit did not arise *via* proteolytic cleavage of the 57 kD subunit during tissue extraction and enzyme purification. The cyanogen bromide peptide maps of the 57 and 55 kD subunits are similar, but non-identical. The APase's native M_r is about 100 kD; this suggests that the native enzyme might exist either as a homodimer or a heterodimer. The heat stable APase has a pH optimum of 5.8, is activated 40% by 4 mM $MgCl_2$, but is unaffected by okadaic acid. Inhibitors include vanadate (I_{50}=3 μM), MoO_4, Pi and NaF. Although activity is observed with a variety of phosphorylated compounds, the specificity constant (i.e. V_{max}/K_m) for *O*-P-Tyr (K_m=0.99 mM) is 3-fold greater than the value obtained for the next best nonartificial substrate (*O*-P-Ser). In addition, this APase dephosphorylates potato tuber P-Tyr-proteins *in vitro*. These data suggest that this APase could function *in vivo* as a P-Tyr-protein phosphatase.

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PURIFICATION OF A MAJOR LEAF ACID PHOSPHATASE WHICH IS ELEVATED IN DEPODDED SOYBEAN PLANTS

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Acid phosphatase activity increased 10-fold in soybean leaves following seed pod removal. *in situ* activity staining showed that most of the activity was associated with leaf vascular tissues and epidermal cell layers. Although soybean vegetative storage proteins (VSP) apparently have low levels of phosphatase activity (DeWald et al., 1992, JBC 267:15958-15964) we found that VSP constituted less than 0.5% of the total activity, despite its great abundance in these leaves. Another protein with acid phosphatase activity was purified to homogeneity and had a subunit molecular weight of about 50 kd. Activity staining of isoelectric focusing gels indicated this protein corresponded to most of the activity bands which are elevated in leaves following depodding. Further characterization of the protein is in progress.

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AMYLOPECTIN TYPE I AND II DEFINE 2 INDEPENDENT STEPS FOR THE BUILDING OF THE STARCH GRANULE.

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In a genetic background where the building of the intermediate size glucans of amylopectin is strongly inhibited due to mutations in a *Chlamydomonas reinhardtii* gene (*ST-3*) necessary for normal soluble starch synthase activity, amylopectin synthesis becomes entirely dependent on the presence of granule-bound starch synthase (GBSS), the enzyme that is thought to be solely responsible for amylose synthesis. In this background we show that the structure of amylopectin is analogous to that of a novel type of high molecular weight branched polysaccharide (amylopectin type II) that we also find in maize. The synthesis of maize amylopectin type II similarly depends on GBSS. This material could be synthesized *in vitro* from native *Chlamydomonas* starch and requires in addition to granule-bound starch synthase the presence of at least one additional branching enzyme. Gene disruption experiments of the *Chlamydomonas* GBSS structural gene (*ST-2*) establish that synthesis of both polysaccharides are independent of one another and mix during the building of the starch granule.

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BIOCHEMISTRY OF TRANSIENT STARCH ACCUMULATION IN YOUNG TOMATO FRUIT

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The young, developing tomato fruit is characterized by a transient starch accumulation which can contribute up to 50% of the dry weight of the fruit tissue. This transiently accumulated starch may serve as a reservoir of carbohydrate for the later accumulation of soluble sugars in the mature fruit. We have studied the metabolic pathway leading from imported sucrose to ADPG, with emphasis on fructokinase (FK). Sucrose synthase, UDPGPPase and FK activities were temporally correlated with the decrease in starch concentration, indicating coordinated control of starch accumulation. FK was partially purified and showed a Km of 0.18 mM fructose, 0.19 mM ATP and a requirement for Mg. Physiological concentrations of fructose, but not F6P, significantly inhibited both FK and sucrose synthase, suggesting that tomato fruit FK may play a regulatory role in determining assimilate import into developing fruit.

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SEASONAL DYNAMICS OF ENZYMES IN ROOTS OF A PERENNIAL WEED (Euphorbia esula).

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Seasonal fluctuations of storage reserves, starch and sucrose, in roots of leafy spurge (Euphorbia esula) are associated with changes in activities of enzymes which metabolize these compounds. Starch is the major reserve carbohydrate which accumulates to approx 30% of the root dry weight in the fall; thereafter starch content declines to approx. 5% of the dry weight. The activities of enzymes which degrade starch, α-amylase, ß-amylase and starch phosphorylase, also fluctuate seasonally, with peaks of activity coinciding with the winter decline in starch content. The activities of other enzymes in the starch degradative pathway, maltose phosphorylase, α-glucosidase and debranching enzyme, remain unchanged throughout the year. Sucrose is also a reserve carbohydrate which accumulates in roots during the winter and remains abundant until bud emergence in the spring. The activity of sucrose synthase, but not alkaline invertase, is highest in winter roots concomitant with the increase in sucrose. The factors regulating the seasonal pattern of enzyme activity include declining temperature and daylength.